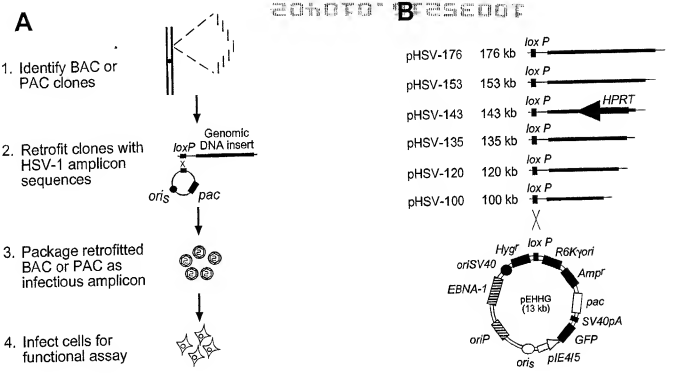


C

Amplicon	pHSV-100	pHSV-120	pHSV-135	pHSV-143	pHSV-153	pHSV-176
Plasmid rescue (bacterial colonies/ $5x10^4$ amplicons)	180	83	360	700	320	33
Intact rescue efficiency	58%	25%	67%	100%	92%	8%

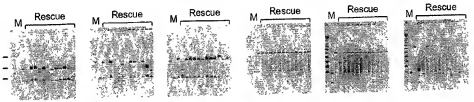


Figure 1

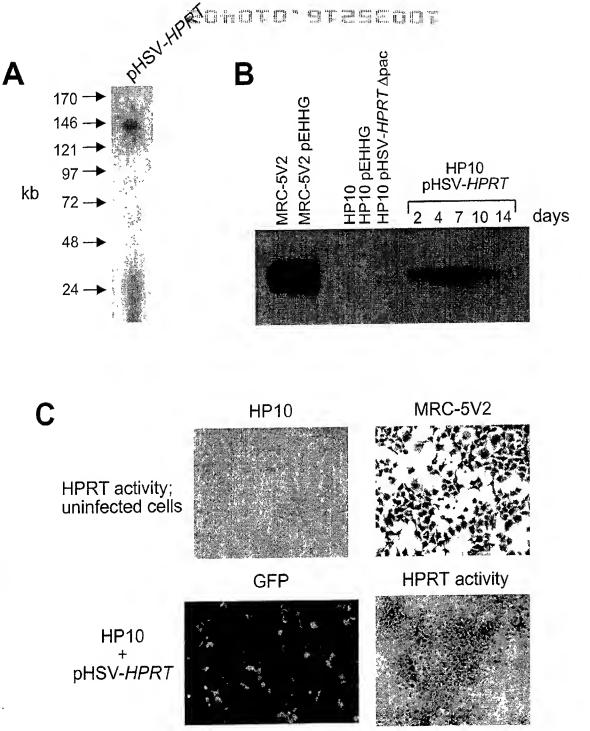

A
pHSV-HPRT
170
146
121
97
kb
72
48
24
B
MRC-5V2
MRC-5V2 pEHHG
HP10
HP10 pEHHG
HP10 pHSV-HPRT Δpac
HP10
pHSV-HPRT
2 4 7 10 14
days
C
HP10
MRC-5V2
HPRT activity;
uninfected cells
GFP
HPRT activity
HP10
+
pHSV-HPRT

Figure 2

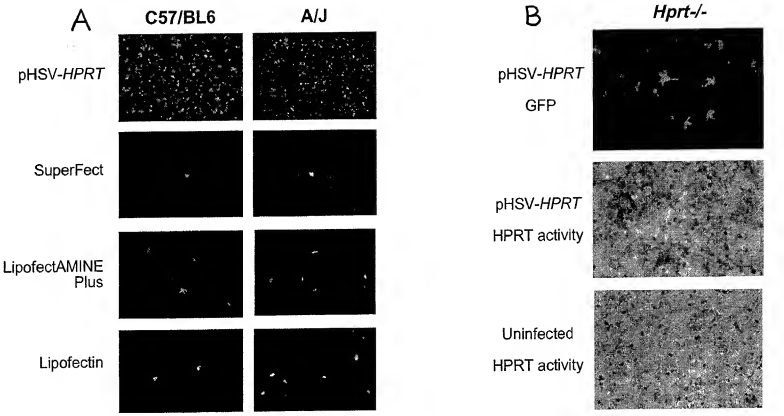


Figure 3

Analysis of pHSV-*HPRT* clonal cell lines

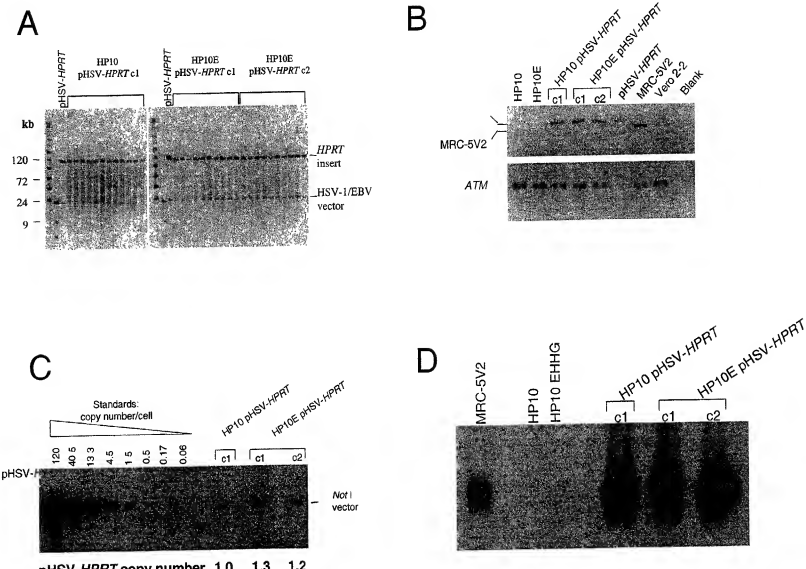


Figure 4

Construction and infectious delivery of *LDLR* BAC genomic DNA vectors

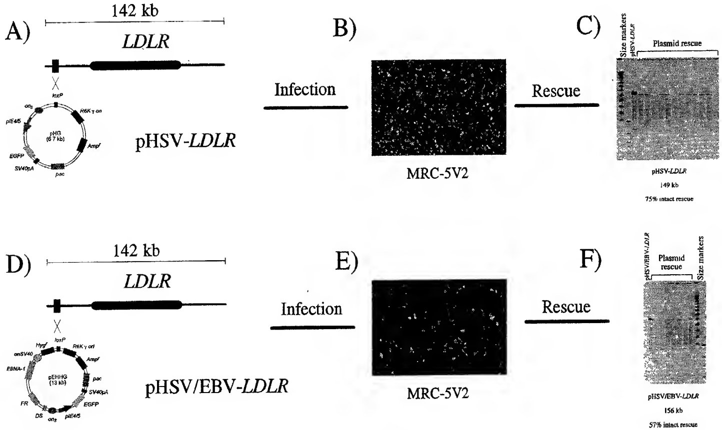


Figure 5

Functional infectious delivery of the human *LDLR* genomic DNA locus

ldlr-/- a7 CHO cells + pHSV-*LDLR* (149 kb)

Figure 6

HsvQuik System

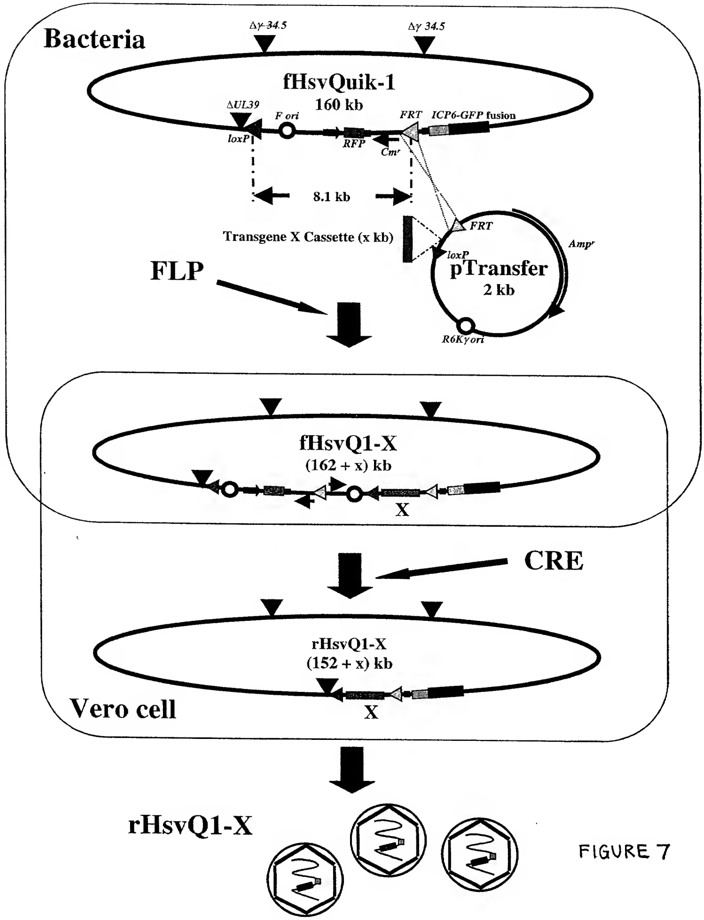


FIGURE 7

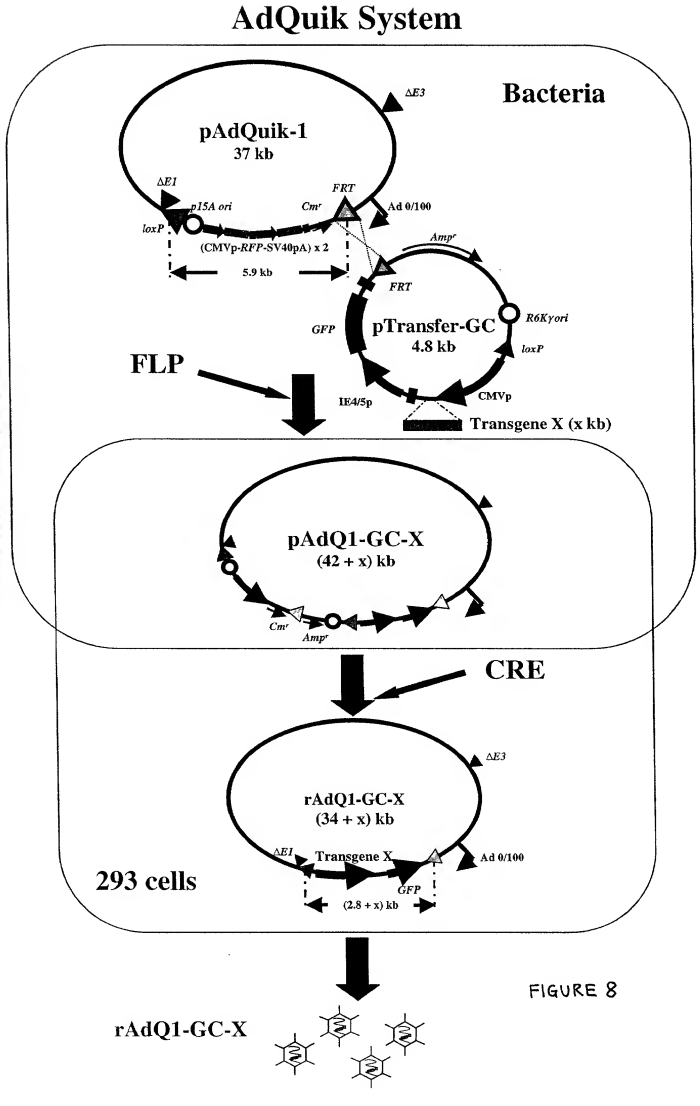
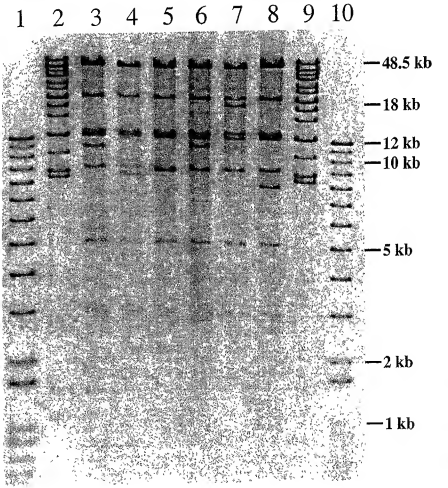


FIGURE 8

Restriction Enzyme (*Hind*III) Digestion Analysis of HSV-BAC Clones



1, 10: 1 kb plus DNA ladder (Gibco/BRL)
2, 9 : H.M.W. DNA ladder (Gibco/BRL)
3 : fHSVQ1-B9
4 : fHSVQ1-C4
5 : fHSVQ1-C6
6 : fHSVQ1-C8
7 : fHSVQuik-1
8 : fHSVQ1

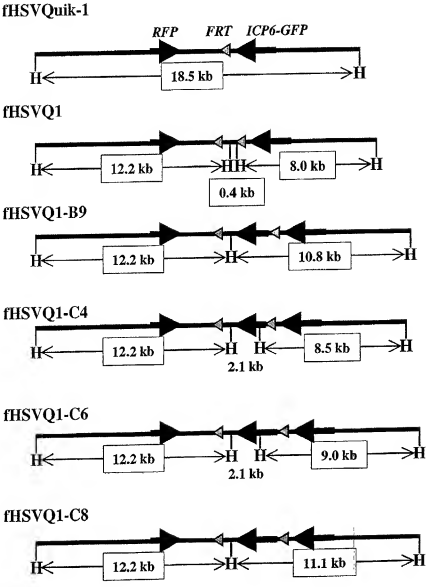
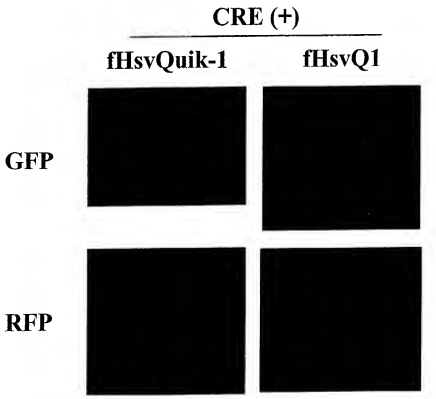


FIGURE 9

Loss of RFP Expression can be Used as an Indicator for Successful Removal of BAC Backbone



VERO cells were cotransfected with pcnCRE and either fHsvQuik-1 or fHsvQ1. Sixty hours later, the progeny viruses were harvested, serially diluted, and inoculate onto VERO cells plated in 96 well-plates. Viral plaques derived from fHsvQuik-1showed both GFP and RFP signals, while those from fHsvQ1 showed GFP signal only. This indicates that the prokaryotic backbone of fHsvQ1 flanked by two *loxP* sites was successfully excised.

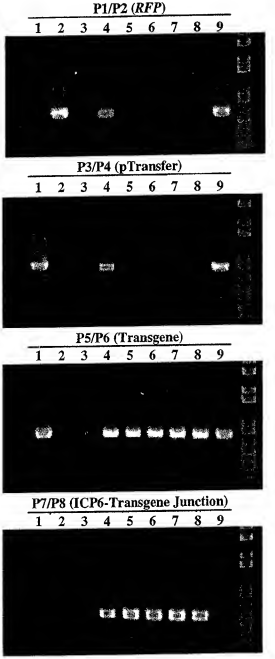
FIGURE 10

Titers of rescued recombinant viruses

	fHsvQuik-1	fHsvQ1
GFP (+) plaques	1.8×10^5	2.0×10^5
RFP (+) plaques	1.8×10^5	2.5×10^3

(PFU/mL)

PCR Analysis of Rescued rHsvQ1s



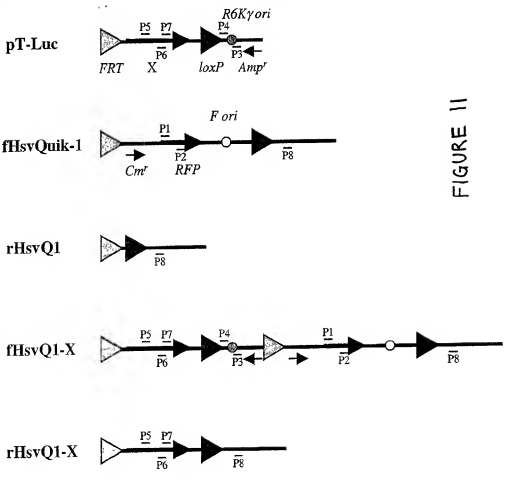
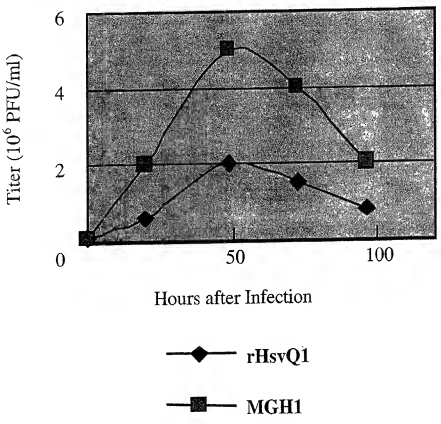


FIGURE 11

1: pT-Luc
2: fHsvQuik-1
3: rHsvQ1
4: rHsvQ1-B9 (RFP+)
5: rHsvQ1-B9
6: rHsvQ1-C4
7: rHsvQ1-C6
8: rHsvQ1-C8
9: fHsvQ1-C8

One-step Growth Curve of rHsvQ1 and MGH1



In vivo safety study on BALB/c mice

Virus: rHsvQ1 v.s. MGH1 (2.5×10^8 pfu/ml)
Animals: 6-week-old female BALB/c mice
(five animals each group)
10 μL injected into right striatum
Duration: 4 weeks
Results: No animal died in either group
$LD_{50} > 2.5 \times 10^6$ PFU

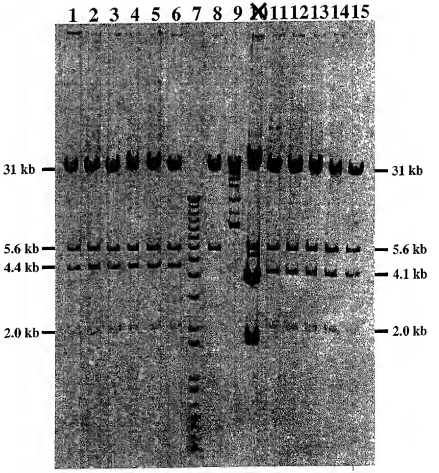
Cytopathic effect *in vitro*

	MGH1	rHsvQ1
Human glioma cell lines		
U87ΔEGFR	6.7 %	19.6 %
Gli36Δ5	18.9 %	20.1 %
U343	49.3 %	44.9 %
Normal human fibroblast		
MRC9	82.7 %	90.3 %

(% surviving cells at 48 hours after infection, moi = 0.1)

FIGURE 12

Restriction Enzyme Digestion Analysis of Mini-Prep DNA



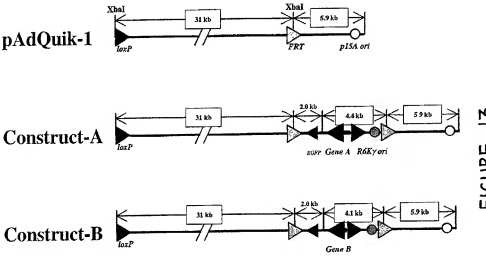


FIGURE 13

Lanes 1-6 : mini-prep (construct-A, XbaI digested)
Lane 7 : DNA ladder (Gibco/BRL)
Lane 8 : pAdQuik-1 DNA (XbaI digested)
Lane 9 : High molecular DNA marker (Gibco/BRL)
Lanes 10-15: mini-prep (construct-B, XbaI digested)

Adenovirus Producing Foci Formation after Transfection

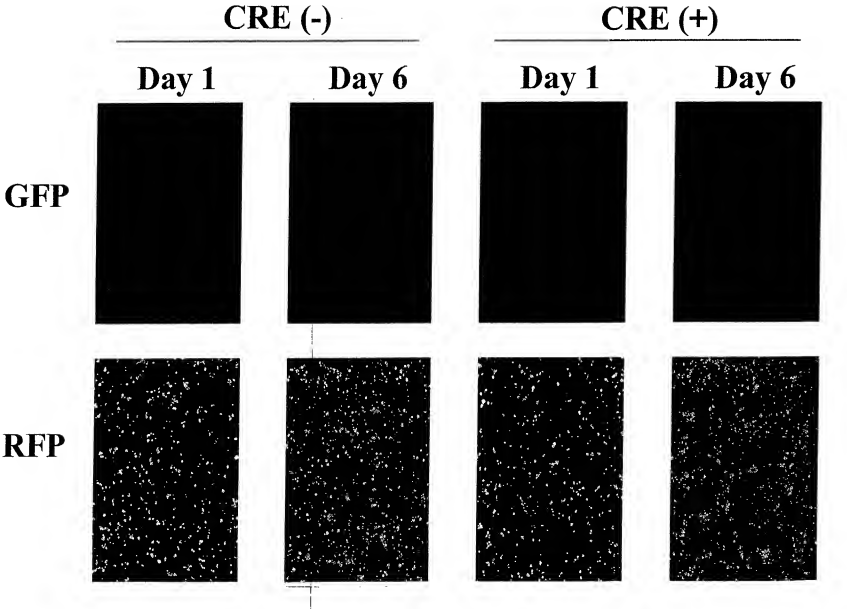
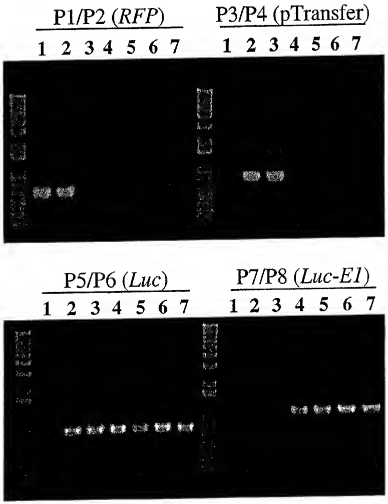


FIGURE 14

PCR Analysis of Rescued rAdQ1-GC-Luc



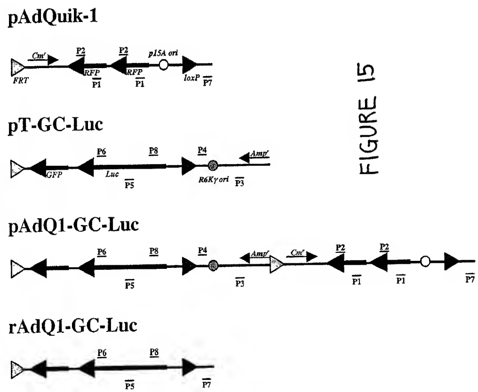
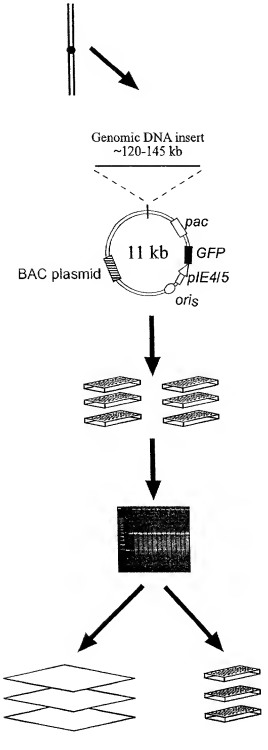


FIGURE 15

Lane 1	: pAdQuik-1
Lane 2	: pAdQ1-GC-Luc
Lane 3	: pT-GC-Luc
Lane 4	: rAdQ1-GC-Luc clone 1
Lane 5	: rAdQ1-GC-Luc clone 2
Lane 6	: rAdQ1-GC-Luc clone 3
Lane 7	: rAdQ1-GC-Luc clone 4

The pHSV-BAC Library I
Library construction and characterization



***Bam* HI partial digestion of chromsomal DNA**
Can make library from any species of interest
eg: human, mouse and rat

Subclone DNA inserts of ~120-145 kb into pHSV-BAC vector

Pick individual clones (approx 200-500,000)
Propagate in 384-well plates, then freeze
Create master library and copies

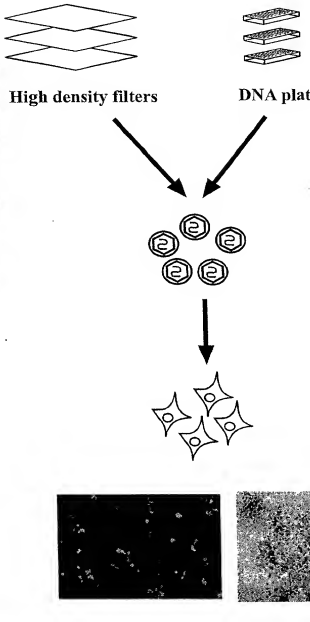
Characterize library
i) Empty clones
ii) Insert size
iii) Insert stability

Build platforms for library screening
i) High density filters
ii) DNA plates

Figure 16

The pHSV-BAC Library II

Screening the library to obtain clones for functional studies



Screen library to identify clones
Two screening platforms will be available.
i) High density filters for hybridization
ii) DNA plates of pooled DNA for PCR

Package clone as HSV-1 vector
Using improved helper virus-free amplicon packaging system.

Infect cell line of interest
Human and mouse cell lines or primary cells may be used

Assay for gene function
In this example GFP and HPRT expression are shown from the pHSV-*HPRT* amplicon in a human fibroblast cell line.

Figure 17

The pHSV-BAC Library III

Library screening by functional assay

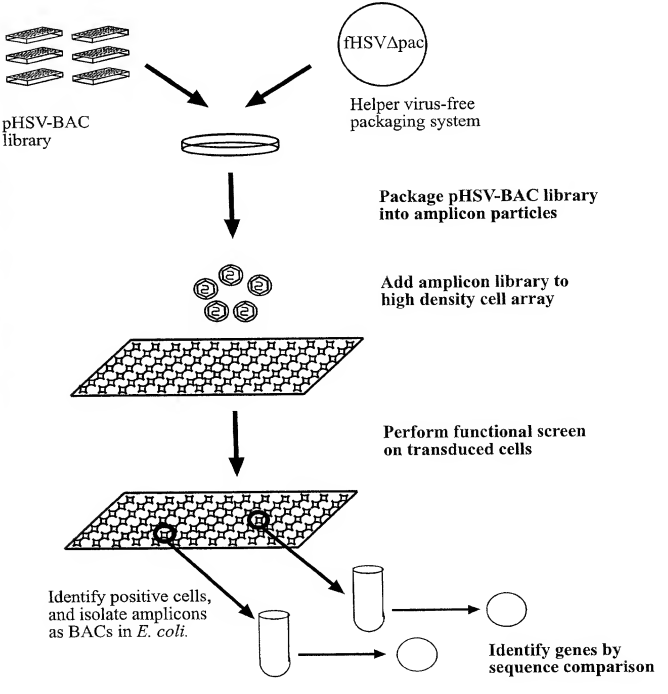


Figure 18